

## Use of agriculture by-products (brans and meal) as food for *Artemia franciscana* (Kellogg, 1906) and effects on performance and biochemical compositions

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### Abstract

Aquaculture needs to provide live food such as *Artemia* and one of the most important issues in *A. franciscana* rearing, is a food supply. The instar I nauplii were fed in seven treatments including Wheat bran, Rice bran, Soy-meal and algae (*Dunaliella salina*). For each treatment calculated growth rate, survival percentage, and body composition. At the reproductive period, 35 pairs were individually isolated from each treatment and transferred to 50 mL Falcon tubes in which the reproduction of females were monitored until their deaths. The result showed that in *Artemia* fed by the agricultural by-product, rice bran ( $10.71 \pm 0.80$ ) and wheat bran ( $10.82 \pm 0.32$ ) obtained the highest growth after control group ( $12.93 \pm 0.16$ ), and the highest survival observed in control ( $56.00 \pm 1.76$ ). Most of the offspring were observed in control ( $896.83 \pm 50.27$ ) and wheat bran ( $880.37 \pm 43.88$ ), but there was no statistical difference between them. Although *A. franciscana* was fed with rice bran similar to the control group in terms of body protein, *Artemia* was fed with wheat bran, significantly increased in body protein. According to the results of this study, it can be concluded that using wheat -and rice bran could be replaced about 90 percent of algae and kept the quality of culturing *A. franciscana* at best.

**Keywords:** *Artemia franciscana*, Brans, Reproductive, Carotenoids, Growth

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## Introduction

*Artemia* is a valuable live food for larval rearing of marine fish and shellfish, that both nauplii and adults with precious materials play a role in meeting the needs of fish food (Sorgeloos 1980; Léger *et al.*, 1986; Sorgeloos *et al.*, 1998; Sorgeloos *et al.*, 2001). Freshly hatched *Artemia* nauplii, in comparison young and adult *Artemia* due to being high in protein and essential fatty acids, have higher nutritional value (Bengtson *et al.*, 1991; Naessens *et al.*, 1997; Dhont and Lavens, 1996; Lim *et al.*, 2001). Adult *Artemia* also has a hormonal substance that will improve reproductive capabilities penaeidae shrimp broodstock (Naessens *et al.*, 1997).

In rearing *Artemia*, food supply is one of the most critical issues. *Artemia* is a non-selective filter feeder, can feed food particles between 1 and 50 microns. Fernandez (2001) revealed that the size of food should be between 6.8 to 27.5 microns for *Artemia* and optimal size is about 16 microns. Extensive cultivation and populations of natural habitats depend on natural microalgae, while in small-scale, semi-intensive and intensive cultivation, used by-products of agricultural and food industry products, including organic fertilizers, rice bran, corn bran, whey, etc. (Wear and Haslett, 1987; Wurtsbaugh and Gliwicz, 2001; Zmora *et al.*, 2002). These diets have contributed to the growth and reproduction of *Artemia* and also decomposed in water, can be fertile

environment and produce natural food such as bacteria, yeast and algae for *Artemia* (Ronsivalli and Simpson, 1987; Brands *et al.*, 1995; Baert *et al.*, 1997; Teresita *et al.*, 2003; Zmora and Shpigel, 2006). Various factors effects on feed behavior *Artemia* such as food filtration rate, the rate of digestion and absorption of food, which they're included quality and quantity of food intake, stage of life and culturing conditions (Coutteau and Sorgeloos, 1989). Although *Artemia* rearing has been successfully conducted by using a wide range of types of microalgae (Vanhaecke and Sorgeloos, 1989), cultivation and preparation of microalgae require high costs, culturing *Artemia* with using mirage is not economically affordable (Sorgeloos, 1982). In the meantime, use of agricultural by-products can be used as an alternative feed the availability in the entire world and also keeps them convenient (Dobbeleir *et al.*, 1980; Zmora and Shpigel, 2006). Research on *Artemia* carried out with agricultural by-products and its impact on growth and survival of *A. urmiana* and parthenogenetic *Artemia* (Ownagh *et al.*, 2015). Wheat and rice bran to possessing fiber can be substituted adequate food in the diet of *Artemia* (Sorgeloos *et al.*, 1980; Piccioni, 1965; Fuller, 2004). On the other hand, soy-meal is a rich source of protein that has a better balance of amino acids and crude fiber content less than 3 percent (McDonald *et al.*, 2002).

This study studied investigations on the effects of agricultural by-products such as wheat bran, rice bran and soy-meal (and mixed them) on *Artemia* reproductive parameters, body composition, and levels of carotenoids. In this survey, we studied the effects of agricultural by-products to the factors mentioned in *A. franciscana* that it is considered an important commercial species in the world.

### Materials and methods

#### *Hatching cysts and rearing nauplii to maturity stage*

*A. franciscana* cysts were hatched under optimal conditions include: water temperature (28°C), salinity (33 ppt), pH (8-8.1) and severe aeration during hatching (Sorgeloos *et al.*, 1980). After hatching, 250 nauplii instar I directly was transferred into flasks of 500 cc (total of 21 containers, each with seven treatments and three repetitions) with 100 ppt salinity. Nauplii density in the first eight days of training was one nauplii per two mL that after of the eighth-day density is dropped to 1 Meta-nauplii per 4 mL of water (Coutteau *et al.*, 1990). Gentle aeration was conducted during the period of rearing in flasks. Some of the water quality parameters, during the period of investigation, were set out as follows: Water temperature: 26±10°C, dissolved oxygen: 7.1-7 ppm and pH 8.3-8.

For all treatments were used photoperiod of 14:10 hours (light/dark). *A. franciscana* was fed following by Coutteau *et al.* (1990). *Dunaliella*

*salina* used as algae. The following treatments considered as an experimental condition: treatment 1 (Wheat bran 90%+10% Algae), treatment 2 (Rice bran 90%+10% Algae), treatment 3 (Soy-meal 90%+Algae 10%), treatment 4 (Wheat bran 45%+Soy-meal 45%+Algae 10%), treatment 5 (Wheat bran 45%+Rice bran 45%+Algae 10%), treatment 6 (Soy-meal 45%+Rice bran 45%+Algae 10%), treatment 7 (100% Algae).

#### *The first phase of the experiment*

Total length of *Artemia* of different treatments was calculated on days of eighth, fourteenth, and twentieth. The nauplii survival rate in different treatments was calculated by calculating the average of the first number of nauplii and adults made in early adulthood (Abatzopoulos *et al.*, 2003).

#### *The second phase of the experiment*

The effect of different diets on reproductive performance and longevity of *A. franciscana* during the maturity period until death.

For surveying reproductive performance and longevity of female in different treatments, 36 pairs *A. franciscana* (in each treatment) were removed from the flasks and placed individually in 50-mL cyllindroconical falcon tubes (36×7=252, number of total individual falcon tubes). At this stage, the production of offspring (cysts and nauplii) was monitored daily in each of the flasks and process continued

until the death of *A. franciscana* female (Abatzopoulos *et al.*, 2003). The recorded reproductive and life span parameters were as follows: total number of offspring, number of nauplii, percentage of encysted embryos, number of broods, days between broods, offspring per brood, offspring per reproductive female in day, pre-reproductive period, reproductive period, post-reproductive period, average maximum and minimum number of offspring per day, survival percentage prior to adult stage, days without producing offspring in adult female and total life span (Abatzopoulos *et al.*, 2003).

#### *The Bacteria analysis*

Bacterial load was measured on the eighth, fourteenth, and twentieth Simultaneous with morphometric measurements. First, 1 mL of the culture media were taken from each flask and was prepared dilution solution (5 times) and were used the last dilution to cultivate with methods of pour plate in the plate count agar. After placing the plates in the incubator at 37°C for 48 hours and counting colonies created, the number of aerobic bacteria calculated and was expressed for log 10 cfu / mL (Arashisara *et al.*, 2004).

#### *Biochemical composition and total carotenoids*

To determine the moisture, protein, lipid, and ash contents of the whole body of *A. franciscana*, proper contents from each treatment were removed and

minced for analysis according to AOAC (2005). Moisture was determined by oven drying at 105°C for 24 h. Crude protein (N×6.25) was determined by the Kjeldahl method after acid digestion using an auto- Kjeldahl System. Crude lipid was determined by the ether-extraction method using Soxhlet System. Ash content was determined using a muffle furnace at 550 °C for six hours (AOAC, 2005).

To calculate of total carotenoids, adult *A. franciscana* (45 mg freeze-dry weight) taken from each treatment and separately were placed in darkness into falcon tube with foil, contain 1.5 mL of pure ethanol (24 hours and at 5°C). Total carotenoids were determined using a spectrophotometer at maximum absorption for each treatment based on the following formula (Moeller *et al.*, 2005).

Total carotenoid ( $\mu\text{g mg}^{-1}$ ) =  $1 \times 10^4$  (OD<sub>450</sub> / 2,620) × (V / W)

OD<sub>450</sub> = optical density at 450 nm (1-cm cuvette)

V = extract volume (mL)

W = total dry mass (mg)

2,620 is the absorption coefficient at 450 nm for a 1% (wt:vol) solution of  $\beta$ -carotene.

#### *Statistical analysis*

Kolmogorov–Smirnov used to check the normality and homogeneity of variances (Triantaphyllidis *et al.*, 1995). The results were analyzed using standard one-way analysis of variance (ANOVA) using SPSS (version 22). Tukey test was applied in order to

determine the significant differences between the means at a significance level of ( $p < 0.05$ ). Charts were drawn with 2013 Excel.

## Results

### Growth and Survival rate

The total length in *A. franciscana* fed by soy-meal had less growth than other *A. franciscana* in treatments ( $p < 0.05$ ). Diets supplemented wheat bran + rice bran along with control during the rearing in the eighth and twentieth days,

showed much higher growth than other treatments and compared to other had significant differences ( $p < 0.05$ ). On the fourteenth day, wheat bran showed a statistically significant difference with treatments were fed with soy-meal ( $p > 0.05$ ) (Fig.1).

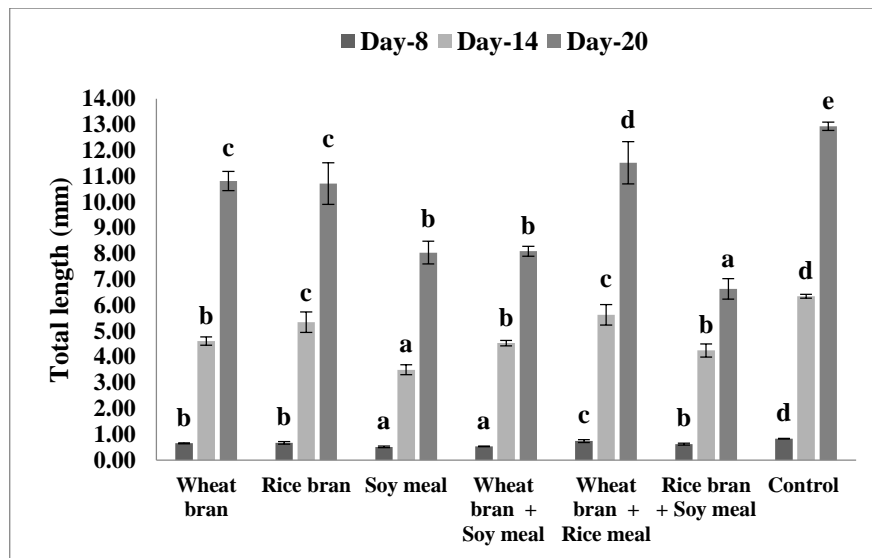


Figure 1: Total length (mm) *A. franciscana*. The comparison did between groups on the same day ( $p < 0.05$ ).

Female survival at the end of the nauplii period, in soy-meal, was the lowest survival and with others were significantly different ( $p < 0.05$ ). Wheat bran+soy-meal was achieved the highest survival females ( $p < 0.05$ ). The

most survival of males related to control ( $21.33 \pm 1.02$ ) and soy-meal was the lowest ( $11.56 \pm 1.02$ ) ( $p < 0.05$ ). Total survival in soy-meal was lower than others ( $p < 0.05$ ) (Table 1).

**Table 1: Mean ( $\pm$ SD) survival (%) of *A. franciscana* reared in different treatments till pre-reproductive period ( $p < 0.05$ ).**

	Wheat bran	Rice bran	Soy meal	Wheat bran + Soy meal	Wheat bran + Rice meal	Rice bran + Soy meal	Control
Survival Rate of female (%)	29.78 $\pm$ 2.14 <sup>ab</sup>	30.44 $\pm$ 1.67 <sup>abc</sup>	27.78 $\pm$ 1.67 <sup>a</sup>	35.11 $\pm$ 2.07 <sup>d</sup>	29.56 $\pm$ 1.39 <sup>ab</sup>	32.22 $\pm$ 1.02 <sup>bc</sup>	33.11 $\pm$ 1.02 <sup>cd</sup>
Survival Rate of male (%)	18.89 $\pm$ 1.02 <sup>c</sup>	18.44 $\pm$ 2.14 <sup>c</sup>	11.56 $\pm$ 1.02 <sup>a</sup>	15.56 $\pm$ 1.67 <sup>b</sup>	16.89 $\pm$ 1.67 <sup>bc</sup>	16.44 $\pm$ 0.77 <sup>bc</sup>	21.33 $\pm$ 0.00 <sup>d</sup>
<b>Total s</b>	48.67 $\pm$ 2.91 <sup>b</sup>	48.89 $\pm$ 3.67 <sup>b</sup>	39.34 $\pm$ 2.40 <sup>a</sup>	50.67 $\pm$ 2.91 <sup>b</sup>	46.44 $\pm$ 2.04 <sup>b</sup>	48.67 $\pm$ 1.76 <sup>b</sup>	56.00 $\pm$ 1.76 <sup>c</sup>

### Bacteria load

Although bacterial load (mean $\pm$ SD) in soy-meal and wheat bran+rice bran in the twentieth day showed the lowest ( $p < 0.05$ ), the highest bacterial load on

the eighth, fourteenth and twentieth days related to wheat bran (36.33 $\pm$ 1.16), rice bran+soy-meal (40.00 $\pm$ 1.73), and control (46.89 $\pm$ SD), respectively ( $p < 0.05$ ) (Table 2).

**Table 2: Mean ( $\pm$ SD) of bacteria loading ( $\times 10^5$ ) of *A. franciscana* cultured in different conditions ( $p < 0.05$ ).**

Bacteria loading	Wheat bran	Rice bran	Soy meal	Wheat bran + Soy meal	Wheat bran + Rice meal	Rice bran + Soy meal	Control
Day-8	36.33 $\pm$ 1.16 <sup>d</sup>	33.34 $\pm$ 1.53 <sup>c</sup>	28.67 $\pm$ 1.15 <sup>a</sup>	30.33 $\pm$ 1.47 <sup>b</sup>	29.68 $\pm$ 1.62 <sup>a</sup>	34.32 $\pm$ 0.58 <sup>c</sup>	34.65 $\pm$ 1.38 <sup>c</sup>
Day-14	39.67 $\pm$ 0.58 <sup>cd</sup>	38.00 $\pm$ 1.00 <sup>c</sup>	31.34 $\pm$ 0.56 <sup>a</sup>	34.23 $\pm$ 1.18 <sup>b</sup>	32.56 $\pm$ 0.49 <sup>a</sup>	40.00 $\pm$ 1.73 <sup>d</sup>	38.69 $\pm$ 2.08 <sup>c</sup>
Day-20	44.58 $\pm$ 1.35 <sup>d</sup>	40.61 $\pm$ 0.85 <sup>c</sup>	34.69 $\pm$ 1.55 <sup>a</sup>	36.43 $\pm$ 1.50 <sup>b</sup>	35.22 $\pm$ 0.98 <sup>a</sup>	44.68 $\pm$ 1.06 <sup>d</sup>	46.89 $\pm$ 1.46 <sup>e</sup>

\* Letters (a,b,c,...) in each raw demonstrated significantly.

### Reproductive and lifespan characteristics

The *A. franciscana* used by soy-meal or soy diets mixed, produced fewer offspring than other ( $p < 0.05$ ), while the most offspring were found in control (896.83 $\pm$ 50.27) and wheat bran (880.37 $\pm$ 43.88). Several rate of nauplii in soy-meal (160.54 $\pm$ 92.64) was lower than others, but the highest amount of nauplii was seen in the control (725.28 $\pm$ 51.39) and wheat bran (729.63 $\pm$ 38.28) ( $p < 0.05$ ). Also, some offspring in *A. franciscana* fed by with rice bran + wheat bran produced fewer than when brans used as single

( $p < 0.05$ ). Percentage of encysted embryos, days between spawning pre-reproductive period and post-reproductive period did not show significant differences in treatments ( $p > 0.05$ ). The number of broods was highest in wheat bran, rice bran, and rice bran + wheat bran ( $p < 0.05$ ). Most of the offspring was in control, and the lowest was in soy-meal. Soy-meal and wheat bran had lowest and highest longevity, respectively ( $p < 0.05$ ) (Table 3).

The number of offspring per brood on *A. franciscana*, in all treatments, showed significant differences

( $p < 0.05$ ), so that were produced in control with the highest ( $130.16 \pm 25.14$ ) and soy-meal with the lowest ( $53.52 \pm 10.98$ ). Many offspring per female per day revealed the same as some offspring per brood almost, the difference was in soy-meal+rice bran and wheat bran+rice bran showed no significant difference ( $p > 0.05$ ) (Table 3).

Production of offspring in females during reproduction was achieved the highest rate of average production in the first 40 days (except soy-meal for the sake of it seen in the first three weeks). The most production females, based on charts, were in the middle of the period of reproduction (Fig. 2).

**Table 3: Mean ( $\pm$ SD) of reproductive characteristics and longevity of *A. franciscana* under different conditions of feeding over the experimental period ( $p < 0.05$ ).**

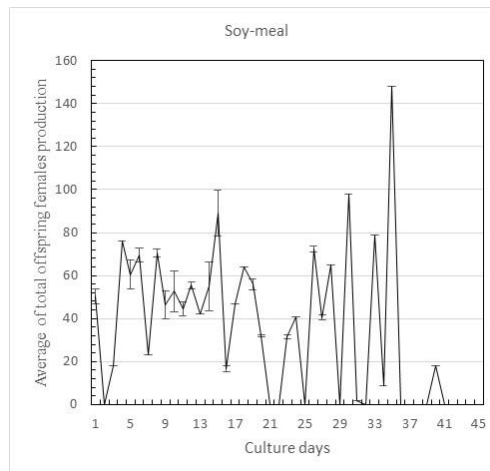
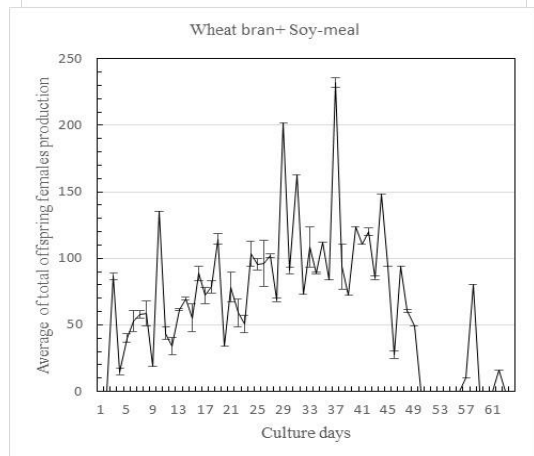
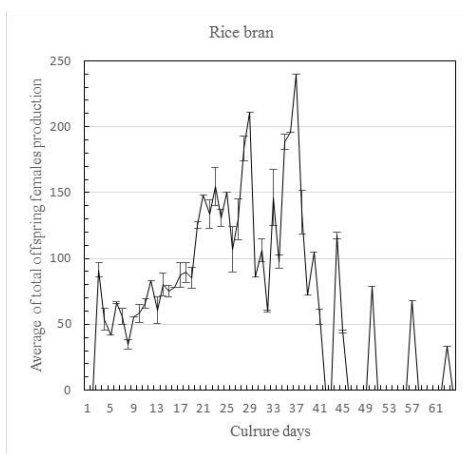
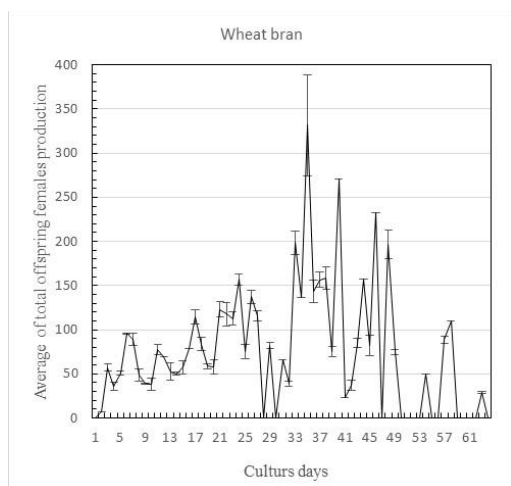
Characters	Wheat bran	Rice bran	Soy meal	Wheat bran + Soy meal	Wheat bran + Rice meal	Rice bran + Soy meal	Control
Total number of offspring	880.37 $\pm$ 43.88 <sup>d</sup>	728.83 $\pm$ 40.63 <sup>cd</sup>	226.39 $\pm$ 11.37 <sup>a</sup>	556.68 $\pm$ 34.90 <sup>bc</sup>	497.88 $\pm$ 35.05 <sup>bc</sup>	372.16 $\pm$ 25.74 <sup>ab</sup>	896.83 $\pm$ 50.27 <sup>d</sup>
Number of nauplii	729.63 $\pm$ 38.28 <sup>d</sup>	622.90 $\pm$ 35.82 <sup>cd</sup>	160.54 $\pm$ 92.64 <sup>a</sup>	476.53 $\pm$ 32.51 <sup>bcd</sup>	398.31 $\pm$ 30.03 <sup>abc</sup>	325.90 $\pm$ 25.76 <sup>ab</sup>	725.28 $\pm$ 51.39 <sup>d</sup>
Percentage of encysted embryos (%)	17.57 $\pm$ 12.07 <sup>a</sup>	16.66 $\pm$ 12.00 <sup>a</sup>	29.91 $\pm$ 13.27 <sup>a</sup>	17.64 $\pm$ 14.81 <sup>a</sup>	22.24 $\pm$ 26.17 <sup>a</sup>	18.53 $\pm$ 18.81 <sup>a</sup>	24.44 $\pm$ 18.94 <sup>a</sup>
Number of broods	8.79 $\pm$ 2.76 <sup>d</sup>	7.50 $\pm$ 2.64 <sup>c</sup>	4.23 $\pm$ 1.36 <sup>a</sup>	7.21 $\pm$ 2.59 <sup>bcd</sup>	6.07 $\pm$ 2.41 <sup>bc</sup>	5.58 $\pm$ 2.15 <sup>ab</sup>	6.89 $\pm$ 2.00 <sup>bc</sup>
Days between spawning	4.95 $\pm$ 1.18 <sup>a</sup>	5.45 $\pm$ 1.78 <sup>a</sup>	5.32 $\pm$ 1.31 <sup>a</sup>	5.71 $\pm$ 1.62 <sup>a</sup>	6.15 $\pm$ 2.05 <sup>a</sup>	5.45 $\pm$ 1.59 <sup>a</sup>	5.86 $\pm$ 1.72 <sup>a</sup>
Reproductive period (days)	45.84 $\pm$ 12.23 <sup>c</sup>	40.44 $\pm$ 10.56 <sup>bc</sup>	25.08 $\pm$ 8.39 <sup>a</sup>	39.53 $\pm$ 13.65 <sup>bc</sup>	38.00 $\pm$ 12.30 <sup>bc</sup>	32.11 $\pm$ 11.51 <sup>ab</sup>	40.83 $\pm$ 11.39 <sup>bc</sup>
Post-reproductive period (days)	3.06 $\pm$ 2.49 <sup>a</sup>	2.61 $\pm$ 3.72 <sup>a</sup>	2.08 $\pm$ 1.71 <sup>a</sup>	1.74 $\pm$ 1.73 <sup>a</sup>	2.19 $\pm$ 2.93 <sup>a</sup>	2.21 $\pm$ 1.81 <sup>a</sup>	1.45 $\pm$ 1.34 <sup>a</sup>
Per-reproductive period (days)	13.74 $\pm$ 0.87 <sup>a</sup>	13.67 $\pm$ 0.84 <sup>a</sup>	13.70 $\pm$ 0.86 <sup>a</sup>	13.74 $\pm$ 0.87 <sup>a</sup>	13.75 $\pm$ 0.86 <sup>a</sup>	13.79 $\pm$ 0.86 <sup>a</sup>	13.83 $\pm$ 0.86 <sup>a</sup>
Offspring per female per day	47.64 $\pm$ 16.75 <sup>b</sup>	45.82 $\pm$ 13.13 <sup>b</sup>	26.27 $\pm$ 9.05 <sup>a</sup>	36.63 $\pm$ 13.91 <sup>ab</sup>	37.29 $\pm$ 16.35 <sup>ab</sup>	30.95 $\pm$ 12.91 <sup>a</sup>	63.21 $\pm$ 28.81 <sup>c</sup>
Maximum-minimum mean offspring production (days)	94.06 23.00	75.05 24.38	39.00 9.00	63.45 15.20	54.90 15.67	61.69 13.80	130.83 29.50
Female:Male ratio	1.58 $\pm$ 0.09	1.67 $\pm$ 0.13	2.41 $\pm$ 0.19	2.27 $\pm$ 0.24	1.76 $\pm$ 0.21	1.96 $\pm$ 0.04	1.45 $\pm$ 0.09
Average number of days without offspring production (%)	23.44	31.25	33.33	21.89	41.54	29.82	26.90
Offspring per brood	100.16 $\pm$ 21.14 <sup>d</sup>	97.18 $\pm$ 19.69 <sup>d</sup>	53.52 $\pm$ 10.98 <sup>a</sup>	77.21 $\pm$ 16.95 <sup>c</sup>	82.02 $\pm$ 17.18 <sup>c</sup>	66.70 $\pm$ 12.78 <sup>b</sup>	130.16 $\pm$ 25.14 <sup>d</sup>
Longevity (days)	59.78 $\pm$ 12.36 <sup>c</sup>	54.11 $\pm$ 10.51 <sup>bc</sup>	38.77 $\pm$ 8.28 <sup>a</sup>	53.26 $\pm$ 13.97 <sup>bc</sup>	51.75 $\pm$ 12.16 <sup>bc</sup>	45.90 $\pm$ 11.86 <sup>ab</sup>	54.67 $\pm$ 11.36 <sup>bc</sup>

\* Letters (a,b,c,...) in each row demonstrated significantly.

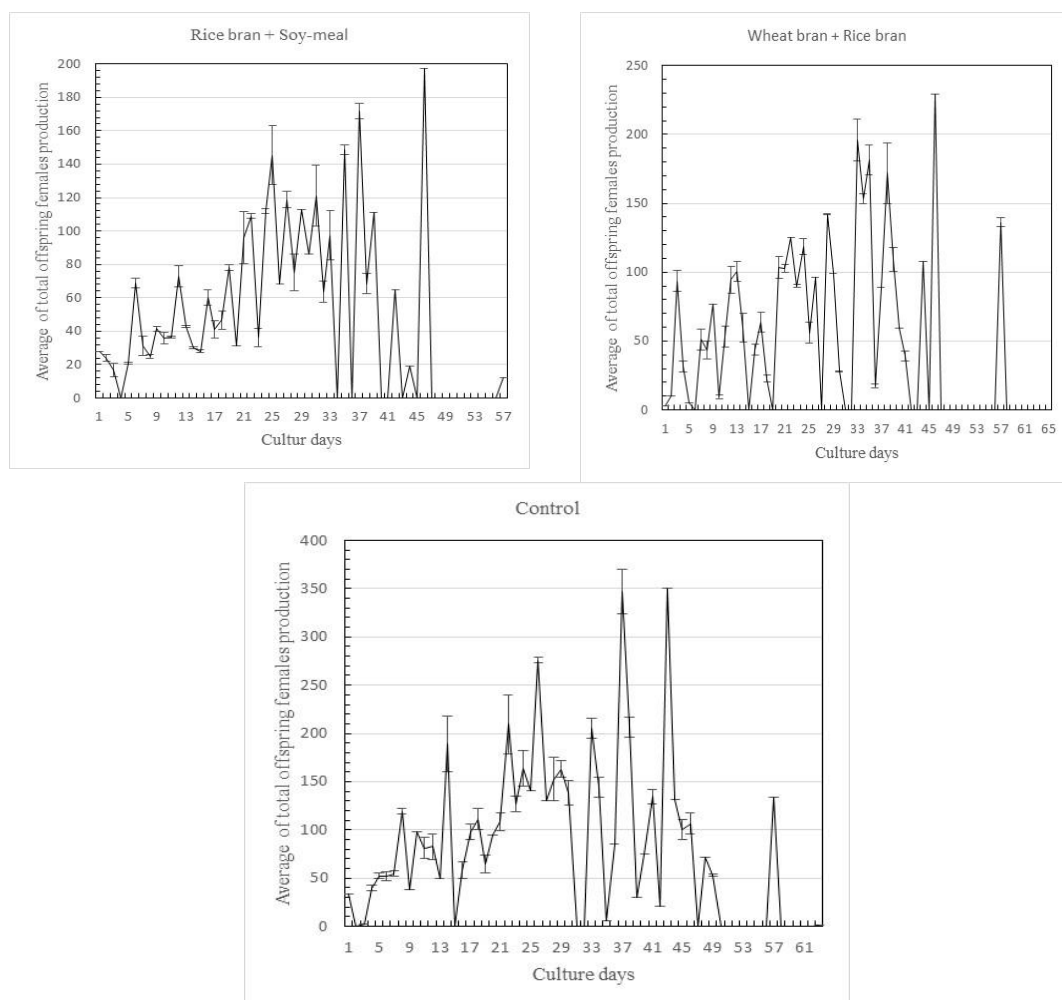
### Biochemical compositions and carotenoids

The total protein content in *A. franciscana* fed with soy-meal ( $50.69 \pm 0.41$ ) showed lowest amount but wheat bran ( $59.97 \pm 0.30$ ) demonstrated the highest amount, respectively ( $p < 0.05$ ). The total protein content in rice bran and control showed no significant difference ( $p > 0.05$ ). No significant in total proteins were observed between wheat bran+rice bran and bran rice+soy-meal ( $p > 0.05$ ). The fat content in control and rice bran showed no significant difference ( $p > 0.05$ ) while the amount of total fat

among the other was significantly different at all ( $p < 0.05$ ). The highest amount of fat was observed in rice bran+wheat bran ( $17.80 \pm 0.56$ ). Although by decreasing fiber in the single diets made increased ash, in control, it was in the lowest ( $p < 0.05$ ). Some total carotenoids with reducing algae dropped in *Artemia franciscana*, only in wheat bran+soy-meal ( $4.88 \pm 0.14$ ) was higher than others and showed significantly different ( $p < 0.05$ ). The highest total carotenoid ( $46.27 \pm 0.11$ ) was observed in control ( $p < 0.05$ ) (Table 4).







**Figure 2: Mean ( $\pm$ SD) female offspring production over the experimental period of *Artemia franciscana* at different feeding regimes.**

**Table 4: Mean ( $\pm$ SD) biochemical body composition and carotenoids in *Artemia franciscana* at different treatments ( $p < 0.05$ ).**

Items	Treatments						
	Wheat bran	Rice bran	Soy meal	Wheat bran+ Soy bran	Wheat bran+ Rice bran	Rice bran+ Soy meal	Control
C.P (%)	59.97 $\pm$ 0.30 <sup>e</sup>	58.98 $\pm$ 0.73 <sup>d</sup>	50.69 $\pm$ 0.41 <sup>a</sup>	53.73 $\pm$ 0.24 <sup>b</sup>	55.97 $\pm$ 0.13 <sup>c</sup>	55.80 $\pm$ 0.85 <sup>c</sup>	58.45 $\pm$ 0.60 <sup>d</sup>
E.E (%)	12.56 $\pm$ 0.20 <sup>b</sup>	11.10 $\pm$ 0.15 <sup>a</sup>	16.84 $\pm$ 0.24 <sup>e</sup>	13.21 $\pm$ 0.09 <sup>c</sup>	17.80 $\pm$ 0.56 <sup>f</sup>	15.65 $\pm$ 0.32 <sup>d</sup>	10.90 $\pm$ 0.23 <sup>a</sup>
Ash (%)	16.00 $\pm$ 0.58 <sup>d</sup>	15.44 $\pm$ 0.36 <sup>d</sup>	18.23 $\pm$ 0.74 <sup>e</sup>	12.86 $\pm$ 0.35 <sup>b</sup>	13.02 $\pm$ 0.47 <sup>b</sup>	14.16 $\pm$ 0.45 <sup>c</sup>	9.73 $\pm$ 0.22 <sup>a</sup>
Moisture (%)	84.44 $\pm$ 0.20 <sup>a</sup>	85.44 $\pm$ 0.42 <sup>b</sup>	86.99 $\pm$ 0.74 <sup>d</sup>	86.26 $\pm$ 0.25 <sup>c</sup>	85.12 $\pm$ 0.72 <sup>ab</sup>	87.13 $\pm$ 0.73 <sup>d</sup>	84.31 $\pm$ 0.33 <sup>a</sup>
Car ( $\mu$ g/mg)	4.50 $\pm$ 0.31 <sup>b</sup>	4.58 $\pm$ 0.22 <sup>c</sup>	4.11 $\pm$ 0.07 <sup>a</sup>	4.88 $\pm$ 0.14 <sup>c</sup>	4.32 $\pm$ 0.29 <sup>ab</sup>	4.25 $\pm$ 0.06 <sup>ab</sup>	46.27 $\pm$ 0.11 <sup>d</sup>

C.P: Crud protein; E.E: Ether extraction; Car: Carotenoids. \* Letters (a,b,c,...) in each row demonstrated significantly.

## Discussion

Since culturing algae need for high capital, so replacement of affordable

food can be one of the most critical solutions in the rearing *A. franciscana*, variety of diets, including low-cost

agricultural by-products (such as rice bran, soy-meal, wheat bran, the whey, etc.) is more acceptable for culturing *Artemia* (Dobbeleir *et al.*, 1980). Researchers on the application of these by-products to grow and mature of *Artemia* is conducted (Sorgeloos *et al.*, 1980), however this diet could effect on *A. franciscana* reproduction and longevity. In this study, we intended to examine reproductive characteristics and lifespan of *A. franciscana* in feeding rice bran, wheat bran, soy-meal, and a mixture of organic materials.

The results of this study proved that the use of agricultural by-product could replace with algal. The growth rate of nauplii showed the highest growth related to algae. Mixed rice and wheat brans compared to others except for algae, had the highest growth. It seems that the combination of these brans reduces the adverse side effects of each of wheat bran and rice bran on the growth of *Artemia* and lack of nutrients needed for growth. The lowest growth was observed in a soy-meal. In terms of food, soy-meal containing raffinose and stachyose that cause bloating, digestive transit rate increase, and digestion and absorption of nutrients reduce. These oligosaccharides are reduced energy resources nitrogen metabolism, digestion of fiber and nutrients passing the time (Coon *et al.*, 1990; Zuo *et al.*, 1996; Parsons *et al.*, 2000). In this study, observed the average growth 12.93, 11.52, 10.82, 10.71, 8.09, 8.04 and 6.64 mm for *A. franciscana* fed by algae, mixed

rice+wheat brans, wheat bran, rice bran, mixed wheat bran+soy-meal, soy-meal and mixed rice bran+soy-meal respectively, it was higher than Terestia and Leticia (2004) had reported rearing *Artemia franciscana* with rice bran and *Tetraselmis suecica* within 15 days arrived to 5.24mm. Anh *et al.* (2009) reported in ponds, use different food sources on the fourteenth day of culture, length reached 8.8 to 9.4 mm that there was not so much difference with this study. Ownagh *et al.* (2015) showed a survival rate for *Artemia Urmiana* with *D. Salina*, wheat bran, soybean, and soybean + wheat bran was 86.3, 70.3, 58.6 and 69.53% on day 15 respectively, while survival rates for *Artemia parthenogenetica* were lower (except for soybean). *Artemia Urmiana* grew more than Parthenogenetic *Artemia*, and maximum growth was 7.82 mm.

Although wheat bran has a high in fiber (Crude fiber content of NDF, ADF and a small amount of ADL) (Feedipedia, 2011), has limited use of wheat bran in diets omnivorous and herbivorous fish (Hertrampf and Piedad-Pascual, 2000). On the other hand, wheat bran contains Pentosans, a substance with antifeedant activity, which reduced the use of nutrients and thus, growth (Choct and Annison, 1992). Allen *et al.* (1994) have been reported wheat with bran has low fat, and there is no problem of corruption in animals in live food. Wheat bran proposed for shellfish between 2-5%, which can be depending on the species,

its value is increased or decreased. Therefore, Wheat bran cannot be a portion of appropriate food for *Artemia* (Hertrampf *et al.*, 2000). In the post-larval blue shrimp *Penaeus stylirostris*, a diet that contained 22% wheat bran with live nauplii, obtained the best growth and survival (Fenucci *et al.*, 1984).

Rice bran fiber is low, and its oil has become valuable food for animals in live food (Göhl, 1982). The survival rate of 72-79% has been observed for *Artemia* fed on live and green algae by Teresita and Leticia (2004) and Dhont and Lavens (1996). Agh *et al.* (2008a) did a study on the growth and survival characteristics of six different populations of *Artemia* in Iran that under different salinities, *Artemia* was fed by using of *Dunaliella tertiolecta* and yeast covered Lansy PZ. at the end of the fourteenth day, the survival rate for urmiana bisexual *Artemia* strain, parthenogenetic Incheh Lagoon, the Salt Lake Qom, ponds around Urmia lake and Varmal lake were 75, 70, 40, 85, 93 and 48% respectively and average growth was 7 -8 mm at the end of the period. Naegel (1999) found survival rates of 72%, 79% and 73.5% for *A. franciscana* cultured for 11 days using commercial inert diets of Nestum (a baby food), enriched Nestum and microalgae *Chaetoceros* sp. (At a density of 2 organisms/mL in a 10-liter bottle) respectively. Rearing of San-Francisco *Artemia* strain from nauplii to maturity in ponds rich on manure and mineral fertilizers, it was found that at

the time of intensive cultivation female *Artemia* length was 7 mm and after catching *Artemia* and reducing density, adult *Artemia* length reached to 15 mm (Rosowski, 1989). In the present study were obtained survival rate 39 to 56%, and the average length between 6.8 to 13 mm in different treatments, which is consistent with mentioned studies. There is increasing evidence that bacteria may play an important role not only in the regeneration and consumption of dissolved nutrients in the water column but also as a food source for direct utilization with herbivorous zooplankton (Rieper, 1978). Early attempted to rearing *Artemia* on diets consisting solely of bacteria failed (D'Agostino, 1980).

However, according to previous studies, it has been made clear that *Artemia* fed on bacteria, but kind of bacterial community in the medium *Artemia* is paramount importance. Intriago and Jones (1993) mentioned *Flexibacter* sp. Was as a food source for the growth and survival *Artemia* and although could play a vital role and had better digest algae in the digestive tract *Artemia*, because of the mismatch increase bacteria with the growth performance, maybe bacteria were grown in these treatments not suitable nutrition as food for *Artemia* and no effect on growth *Artemia*. Length and survival high in this study (control) could be due to existence beneficial bacteria and help them to better digestion of algae in the digestive tract

*Artemia* have been that confirmed by Intriago and Jones (1993).

According to the results, total production of offspring (except algae), in wheat bran was higher than others, and the differences were statistically with algal (Basil *et al.*, 1995). Anh *et al.* (2009) in his studies have noted that the rate of fecundity in *Artemia* is affected by diets, it is concluded that wheat bran has better performance than other bran in reproductive *Artemia* and reproduced more frequently. Several studies have shown that reproduction model of *Artemia* is influenced by many factors such as salinity, temperature, photoperiod length, species and geographical strain *Artemia*, salt-water conditions, quality and quantity of food, genetics and other environmental factors (Triantaphyllidis *et al.*, 1995; Browne and Wanigasekera, 2000; Abatzopoulos *et al.*, 2003; Baxevanis *et al.*, 2004; El-Bermawi *et al.*, 2004).

Dwivedi *et al.* (1980) found that using yeast as food; about 31% of females did oviparity while algae did it about 23%. Versichele and Sorgeloos (1980) reported that diets containing rice bran and *spirulina*, are stimulated *Artemia* to oviparity, whereas *Scenedesmus* sp. also *Dunaliella* sp. are less stimulating. Percentage of encysting of the embryo in *Artemia* fed by *Dunaliella* Salina as compared to rice bran was higher, and the results do not match with studies Versichele and Sorgeloos (1980), although no

statistically significant difference was observed between treatments.

Much research is done on reproductive features and longevity of bisexual and parthenogenetic *Artemia* populations from different geographical areas. In these studies, it was found that among different populations, environmental conditions and water physicochemical factors, there are differences in areas, features reproductions, lifespan and reproductive periods; however, in a series of characteristics were similar to each other. According to Triantaphyllidis *et al.* (1995), although pre-reproductive period and lifespan in the population of Tanggu parthenogenetic *Artemia* and *Artemia franciscana* from San Francisco Bay had no significant difference, the period of reproduction of *Artemia franciscana* was more than parthenogenetic *Artemia*.

The use of rice bran as a source of cheap food and waste material as feed *Artemia* resulted in a relative increase in the amount of crude protein body, also *Artemia* fed on rice bran showed a massive increase in the total amount of amino acids, if *Artemia* can food with low quality converted to proteins with quality (Sorgeloos *et al.*, 1980). Agh and Hosseini Ghatre (2002) recorded adult *A. Urmiana* fed by rice bran had 52.25% protein content. Naegel (1999) revealed protein content 56.4%, 42.87%, and 41.16% for *A. franciscana* fed by Chaetoceros, Nestum, and enrichment Nestum, respectively.

Teresita and Leticia (2004) reported 53.1% protein content for *Artemia* reared on rice bran and *T. suecica*. As is clear from the results of this study, rice bran with algal and wheat bran showed higher values in body protein that is consistent with Sorgeloos *et al.* (1980). Fat and particularly fatty acid profile *Artemia* are related to factors such as type strain, environmental factors and changes in biochemical composition of food (mainly algae available to adult) heavily (Léger *et al.*, 1986). All fats found in rice bran can during the stored wiped out due to enzyme degradation lipolytic when is activated, separating the bran from rice. Rice bran has 14-18% fat (Göhl, 1982). It is likely the reason why *Artemia* body fat in rice bran has been lower than others. Teresita and Leticia (2004) reported ash content 15.4, 19.1, 8.7, 10.77 and 33.9 percent based on their dry weight for *Artemia* reared on rice bran, *T. suecica*, dried *Spirulina*, wet *Spirulina* at day 15 and wild *Artemia* has grown in nature, respectively. Ownagh *et al.* (2015) reported that feeding with soybean showed higher protein for *A. Urmia* (48.8%), and Parthenogenetic *Artemia* (51.1%). Also, wheat bran increases fat and ash in *A. urmiana* (25.02 and 12.21%), and soybean increase fat and ash for Parthenogenetic *Artemia* (11.84 and 13.3%). While these results showed the feeding by algae could decrease ash content (9.73%) on DW. Ash content for *A. franciscana* fed on soy-meal, wheat bran, rice bran, rice bran+soy-meal, wheat bran+ rice bran and wheat

bran+ soy-meal were 18.23, 16.00, 15.44, 14.16, 13.02, and 12.86, respectively. *Artemia*, like other crustaceans, intake carotenoids through food (such as *D. salina* is a vibrant and vital source of beta-carotene) and much of it will be moved to the gonads and cysts. In this study, total carotenoids in *Artemia* body were highest when they consumed on *D. salina*.

Generally, to obtain the highest rate of *A. franciscana* biomass with high survival rates could be used a combination of wheat bran and soy-meal. It seems wheat bran is appropriate to produce high levels of protein in *A. franciscana*. In terms of reproductive performance can be mentioned that wheat bran instead of algae influenced without any significant differences in the reproduction of *A. franciscana*. Also, wheat bran made increasing *A. franciscana* lifespan. Therefore, based on these results, the use of agricultural wastes, which costs are much lower than algae, have not an apparent negative effect than algae on *Artemia*, sometimes the effect could be more favorable.

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